# Biophysical methods for studying human GPCR ligand binding properties

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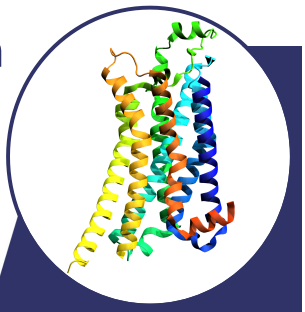
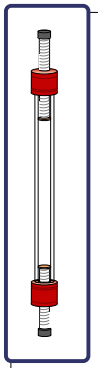


## 1. Introduction

Membrane proteins account for >30% of the human proteome and account for a large proportion of the proteins targeted by pharmacological intervention of human diseases[1]. As such, high-quality membrane protein reagents are highly desirable tools for drug discovery campaigns. However, membrane protein production and purification can be very challenging with a number of difficulties, such as; poor expression, solubilisation and stability. Our aim was to explore ways in which we can overcome these challenges, and validate the quality of expressed and purified protein by demonstrating target engagement by physiological ligands or known small molecule inhibitors. Using GFP-tagged human G-protein coupled receptors (GPCRs), we tested the effect of various conditions on their solubility and stability using rapid and generic biophysical techniques.



## 2. FSEC using crude extract of S1PR1

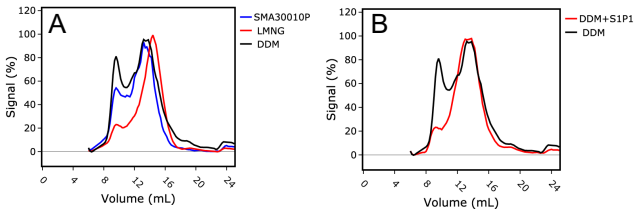
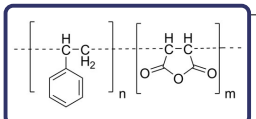



**Figure 1 - FSEC using crude extract of S1PR1 indicates optimal membrane extraction conditions and ligand binding.** Analysis of crudely solubilised GFP-tagged human S1PR1 from insect cell culture used fluorescent size-exclusion chromatography (FSEC). The quality of protein sample is assessed by the monodispersity index; the ratio between protein in the void (~8 mL column retention) compared with monodispersed sample (14-15 mL column retention). A) Comparison of FSEC traces of S1PR1 solubilised in styrene maleic acid co-polymer (SMA; blue), lauryl maltose neopentyl glycol (LMNG; red) or dodecyl maltoside (DDM; black). Sample solubilised in LMNG displayed a superior FSEC profile. B) Comparison of FSEC traces of S1PR1 solubilised in DDM in the presence (red) or absence (black) of the agonist sphingosine-1-phosphate (S1P). Sample solubilised in the presence of S1P showed a superior FSEC trace with reduced aggregation.



## 3. Stabilisation of $5HT_{2A}R$ using SMALP

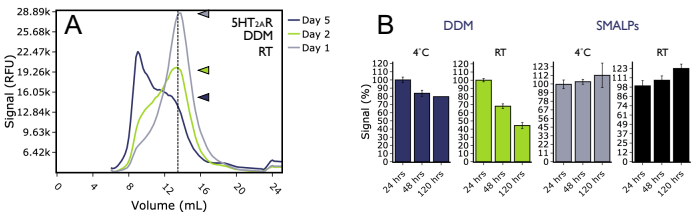
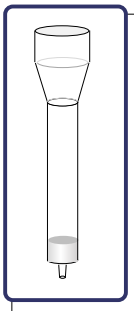



**Figure 2 – Extraction of $5HT_{2A}R$ from the membrane using SMALP provides better long-term stability than detergent.** GFP-tagged human $5HT_{2A}R$ was solubilised from insect cell membranes in either detergent (DDM) or SMA polymer and analysed by FSEC at several time points after storage at 4˚C or room temperature (RT). Following several days incubation, $5HT_{2A}R$ in DDM displayed a significant drop in monomeric peak height at either temperature, whereas $5HT_{2A}R$ in SMALP did not, indicating protein in SMALP remains stable for longer, even at unfavorable temperatures. A) representative FSEC traces of $5HT_{2A}R$ solubilised in DDM and stored at room temperature for one (grey), two (green) or five (blue) days. B) Histograms of normalised monomeric peak height for DDM sample stored at 4˚C (blue) or RT (green) compared to SMALP samples stored at 4˚C (grey) or RT (black).



## 4. Purification of $5HT_{2A}R$ using FLAG-affinity and SEC

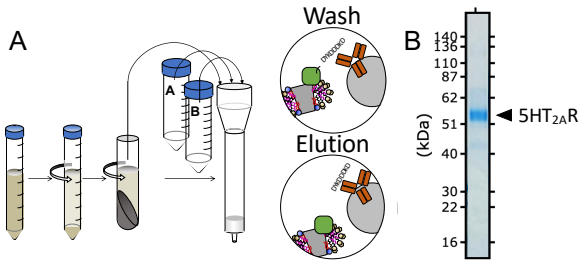
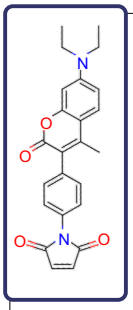



**Figure 3 – Purification of $5HT_{2A}R$ in detergent using FLAG-affinity and SEC purification steps.** GFP-tagged human $5HT_{2A}R$ was solubilised from insect cell membranes in DDM and purified to >95% purity using a two-step purification method. A) Schematic representation of the solubilisation and FLAG-affinity capture steps. B) Representative final purification sample run on Coomassie stained SDS-PAGE following final SEC step.



## 5. Thermal shift assay on $A_{2A}R$ using CPM dye

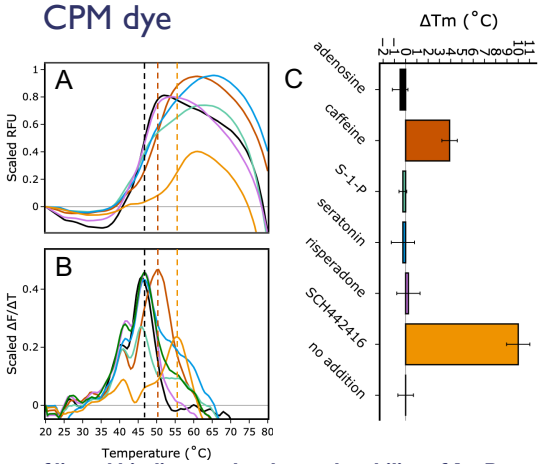



**Figure 4 – Effect of ligand binding on the thermal stability of $A_{2A}R$.** The thermal stability of purified human $A_{2A}R$ in detergent in the presence of different compounds was measured using the thiol-reactive fluorochrome N-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide (CPM)[2]. Raw melt-curves (A) and corresponding first-derivative curves (B) were used to calculate the half-unfolding temperature (Tm) of $A_{2A}R$ in the presence of a panel of different small-molecule compounds at a concentration of 1 mM. C) Histogram of observed thermal stability shifts of $A_{2A}R$ in response to compounds (stability of $A_{2A}R$ in the absence of compound used as baseline). Only when the two specific $A_{2A}R$ antagonists were present (caffeine; brown, or SCH442416; orange) was the stability of $A_{2A}R$ shifted to a higher Tm. This finding demonstrated the properties of purified $A_{2A}R$ were as physiologically expected, thus, validating its fold and activity.

## 6. Conclusions

The generic systematic approaches for condition screening we present here allow us to quickly optimise solubilisation and purification parameters for the production of membrane proteins. This means we can produce stable and functionally active membrane protein for biophysical and structural studies rapidly.

## 7. References

1. Sriram K, Insel PA (2018) G Protein-Coupled Receptors as Targets for Approved Drugs: How Many Targets and How Many Drugs? *Mol Pharmacol* 93(4):251–258.
2. Harborne SPD, King MS, Kunji ERS (2020) Thermostability Assays: a Generic and Versatile Tool for Studying the Functional and Structural Properties of Membrane Proteins in Detergents. Biophysical Journal, pp 105–121.